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Application No. **09/762,850**	Filing Date **April 13, 2001**	Examiner **David M. Naff**	Customer No. **041068**	Group Art Unit **1651**

Invention: **Method For Producing Ultra-Pure Al[illegible]**

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COMBINED TRANSMITTAL OF APPEAL BRIEF TO THE BOARD OF PATENT APPEALS AND INTERFERENCES & PETITION FOR EXTENSION OF TIME UNDER 37 C.F.R. 1.136(a) (Small Entity)	Docket No. 113737.6

In Re Application Of: **Ulrich Zimmermann, et al.**

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/762,850	April 13. 2001	David M. Naff	041068	1651	2752

Invention:

Method For Producing Ultra-Pure Alginates

COMMISSIONER FOR PATENTS:

This is a combined Transmittal of Appeal Brief to the Board of Patent Appeals and Interferences and petition under the provisions of 37 CFR 1.136(a) to extend the period for filing an Appeal Brief.

Applicant(s) hereby request(s) an extension of time of (check desired time period):

☒ One month ☐ Two months ☐ Three months ☐ Four months ☐ Five months

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COMBINED TRANSMITTAL OF APPEAL BRIEF TO THE BOARD OF PATENT APPEALS AND INTERFERENCES & PETITION FOR EXTENSION OF TIME UNDER 37 C.F.R. 1.136(a) (Small Entity)	Docket No. 113737.6

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09/762,850	April 13. 2001	David M. Naff	041068	1651	2752

Invention:

Method For Producing Ultra-Pure Alginates

TO THE COMMISSIONER FOR PATENTS:

This combined Transmittal of Appeal Brief to the Board of Patent Appeals and Interferences and petition for extension of time under 37 CFR 1.136(a) is respectfully submitted by the undersigned:

Signature

Dated: **15 September 2004**

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zimmermann, et. al.	:	Group No.: 1651
Serial No.: 09/762,850	:	Examiner: David M. Naff
Filed: April 13, 2001	:	
For: Method for Producing Ultra-pure Alginates	:	

BRIEF ON APPEAL UNDER 37 C.F.R. § 1.192

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Real party in Interest

Applicant's real party in interest is:

CELLMED AG
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Related Appeals and Interferences

Applicants, Applicants' assignee and Applicants' legal representative are unaware of any appeals or interferences that are related to the instant appeal, or that will affect, be affected by or have any bearing on the Board's decision in the instant appeal.

Status of the Claims

Claims 29 to 42, 52, and 56 are currently pending in the application. Claims 29 to 42, 52 and 56 stand rejected under 35 U.S.C. § 112 first and second paragraphs, and § 103(a). The Claims as currently pending in the application are presented in Appendix A.

As originally filed, the application contained claims 1-28. In a preliminary amendment claims 1-28 were canceled and replaced with claims 29 - 55. Claims 29-42, and 52 were elected without traverse in response to a restriction requirement and non-elected claims 43-51 and 53-55 were cancelled. In response to a first non-final Office Action rejecting all of these Claims, amendments were submitted, and Claim 56 was added.

Claims 29-42, 52, and 56 were rejected in a Final Office Action, and amendments to Claim 29 and 52 were submitted. These amendments were denied entry into the Record. A Request for Continuing Examination was then entered with these amendments, whereby the amendments were entered into the Record.

In a non-final Office Action mailed in the Request for Continued Examination February 6, 2004, claims 29-42, 52, and 56 were rejected under 35 U.S.C. § 112 first and second paragraphs, and § 103(a).

In a supplementary Office Action mailed April 2, 2004, (hereafter referred to as "Supplementary Action") the Examiner reconsidered the Applicants' responses to the Office Action mailed February 6, 2004. The rejections of claims 29-42, 52, and 56 under 35 U.S.C. § 103(a) and the Applicants' responses were reconsidered in light of the Declaration of Dr. Frank Thürmer, submitted on January 29, 2004 ("Thürmer") and the journal publication of Orive, et. al. ("Orive"), contained in an Information Disclosure Statement as item AA, filed March 19, 2004. The Examiner maintained the rejections of claims 29-42, 52, and 56 under 35 U.S.C. § 112, first

and second paragraphs, and § 103(a), stating that the Declaration failed to overcome the previous rejections of all claims.

Applicants appeal from the rejection of claims 29-42, 52, and 56 under 35 U.S.C. § 112, first and second paragraphs, and § 103(a) as maintained in the Supplementary Office Action.

Status of Amendments

No amendments have been made post the First Office Action rejection in the Request for Continued Examination.

Summary of the Invention

Claim 29 on appeal recites a process for obtaining a highly purified alginate composition. Alginates are materials derived from algae and are used, for example, in products intended to be ingested by human beings, such as foods and pharmaceuticals. Clearly, such alginates must be as free as possible of substances which are toxic or otherwise biologically or biochemically harmful to healthy living things. This is accomplished by purification of the alginate, either combined with, or following, extraction. The invention recited in Claim 29 recites a process supported in the Specification (inter alia p. 7, ln. 22 - p. 8, ln. 26; p. 4, ln. 30 - p. 5, ln 34), which produces alginates that have improved purity and biocompatibility compared with alginates prepared by processes in the prior art (inter alia, p. 16, ln. 23 - p. 17, line 9; "Examples of Performance" pp. 12 - 20).

The process of Claim 29 comprises the steps of: a) treating raw algae material with a complex forming agent (also known as a chelating agent) creating a liquid comprising dissolved alginate and solid matter; b) filtering said liquid to produce a filtrate, said filtrate being a

solution comprising dissolved alginate; c) precipitating said alginate out of said solution; d) collecting and dewatering the precipitated alginate; and e) repeating the steps a) to d) at least once.

Claims 30 - 42 and 56 on appeal depend from, and further limit, the process recited in Claim 29. Claims 30 limits the complex forming agent to ethylene diamine tetra acetic acid and Claim 31 limits the extracting (treating) to taking place in a soda solution. Claims 32-35 further define the extraction (treating), sedimentation, and filtering steps. (Regarding the use of the words "extraction", "extracting" and "treating" in the this paragraph, please see Subsection 2D of this Brief, second paragraph.)

Claims 36-39 further define the precipitating, collecting, and dewatering steps. Claims 40-42 and 56 further define the algae material used in the process.

Claim 52 on appeal is a product-by-process claim depending from Claim 29 and reciting an alginate composition and its properties, the composition manufactured by the process according to Claim 29.

Issues Presented for Appeal

1. Whether the Examiner has erred in rejecting Claims 29, 31-42, 52, and 56 under 35 U.S.C. § 112, first paragraph.

2. Whether the Examiner has erred in rejecting Claims 29 - 42, 52, and 56 under 35 U.S.C. § 112, second paragraph.

3. Whether the Examiner has erred in rejecting Claims 29 - 42, 52, and 56 under 35 U.S.C. § 103 (a).

Grouping of Claims

Group I

Independent Claim 29 recites a process for obtaining a highly purified alginate composition. This claim comprises the steps of: a) treating raw algae material with a complex forming agent (also known as a chelating agent) creating a liquid comprising dissolved alginate and solid matter; b) filtering said liquid to produce a filtrate, said filtrate being a solution comprising dissolved alginate; c) precipitating said alginate out of said solution; d) collecting and dewatering the precipitated alginate; and e) repeating the steps a) to d) at least once.

Claims 30 - 42, 52, and 56 depend from Claim 29. Claim 30 limits the complex forming agent to ethylene diamine tetra acetic acid and Claim 31 limits the extracting (treating) to taking place in a soda solution. Claims 32-35 further define the extraction (treating) and filtering steps. Claims 36-38 further define the precipitating step. Claim 39 further defines the dewatering step. Claims 40- 42 and 56 further define the algae material used in the process.

Claim 52 is a product-by-process claim depending from Claim 29 and reciting an alginate composition and its properties, the composition manufactured by the process according to Claim 29.

None of the claims in this group are considered to be patentably distinct from one another.

For ease of review, the claims may be grouped as follows.

Group I: Claims 29-42, 52, and 56.

Argument

The Applicants respectfully submit that the Examiner has erred in rejecting claims 29 to 42, 52 and 56 under 35 U.S.C. § 112, first and second paragraphs and § 103(a). In rejecting claims 29, 31 to 42, 52 and 56 under 35 U.S.C. § 112, first paragraph the Examiner has failed to demonstrate that one of ordinary skill in the art would be required to engage in undue experimentation to practice the invention with complex forming agents other than EDTA. In rejecting claims 29 to 42, 52 and 56 under 35 U.S.C. § 112, second paragraph the Examiner has not applied the objective "person having ordinary skill in the art" test to the claims, and has ignored the clear disclosure of the specification. In rejecting claims 29 to 42, 52 and 56 under 35 U.S.C. § 103(a) the Examiner has improperly modified the Klöck et al reference to eliminate the critical teaching of Klöck et al for the purification of alginates and substituted his own hypotheses regarding portions of the process disclosed by Klöck et al.

In the following, the Applicants present arguments and evidence in support of their position. Some of the evidence is based on the Declaration of Dr. Frank Thürmer, ("Thürmer") entered into the prosecution record under 37 C.F.R. § 1.132 on January 29, 2004, and duplicated in this brief as Appendix B, with page numbers and line numbers added to make citation easier. All citations to this Declaration found in this brief refer to the Appendix B copy. Other evidence will be presented from the journal publication of Orive et. al. ("Orive"), which is item AA in an Information Disclosure Statement entered into the prosecution record on March 19, 2004, and duplicated in this brief as Appendix C.

1. Claim Rejections - 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 29, 31-42, 52, and 56 on the grounds that the use of complex-forming agents other than EDTA is not enabled by the specification. The Applicants respectfully submit that the Examiner is in error. It is submitted that the Examiner does not provide evidence in support of his arguments, relying instead mainly on his own understanding of the present claims. In response, the Applicants respectfully submit that the Examiner has misconstrued Claim 29.

The test for enablement of a claimed invention is whether or not one of ordinary skill in the art can make and use the invention without undue experimentation. *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988).

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 190 USPQ 214 (CCPA 1976).

There are multiple factors to be taken into account in determining if experimentation is undue.

(A) The breadth of the claims;
(B) The nature of the invention;
(C) The state of the prior art:
(D) The level of one of ordinary skill in the art;
(E) The level of predictability in the art;
(F) The amount of direction provided by the inventor;
(G) The existence of working examples; and
(H) The amount of experimentation needed to make or use the invention based on the content of the disclosure.

It is apparently the Examiner's contention that one of ordinary skill in the art in the purification of biological samples is unfamiliar with complex forming agents such as EDTA. The Applicants respectfully submit that one of ordinary skill in the art would be familiar with

complex forming agents and could select alternative complex forming agents to EDTA without undue experimentation. The scope of the claim is not overly broad with respect to the term complex forming agent, and based on the specification and the knowledge of those of ordinary skill in the art, the nature of the function of the complex forming agent is very clear: to remove multivalent cations and allow the alginate to dissolve. Further, Applicants respectfully submit that it is predictable which complex forming agents will be capable of performing this function.

The term "complex forming agent" is known to one of ordinary skill in the art, and examples other than EDTA are likewise known. The use of EDTA is presented as one embodiment of the invention.

Referring to Appendix D, six numbered dictionary definitions are shown, including ones for "complexing agent" (Def. 1), "chelate" (Def. 2 and 5), and "chelation" (Def. 5), all synonymous with, or closely akin to, the term "complex forming agent." See also in Appendix D the dictionary entries for EDTA (Def. 4 and 6) and EDTAN (Def. 3). Please see also Klöck (AP), p. 639, under "Results", first and second sentences, which makes a clear distinction between Ba^{2+} alginate gels and chelating agents such as EDTA.

Other examples of complex forming agents include nitrilotriacetic acid (Def. 1), ethylenediaminetetraacetonitrile (EDTAN) (Def. 3), and ones based on polyaminocarboxyllic acids (Def. 1).

The general manner in which these agents act, namely the binding of the agents to multivalent cations (i.e. positively charged ions with charges of +2 or more), is known and understood (Def. 1, "chelate"). The invention recited in Claim 29 involves a process that exploits this action of complex forming agents in a novel way, supported in the Specification (p. 4, ln. 30 - p. 5, ln 34; p. 7, ln. 21-23), in order to produce alginates that have improved purity and

biocompatibility compared with alginates purified by prior processes (p. 16, ln. 22 - p. 17, line 9).

In further support of these arguments, please see Thürmer, Appendix B, page 5, line 7-11.

Therefore, the Applicants respectfully submit that claims 29, 31-42, 52, and 56 are enabled for complex forming agents other than EDTA. Applicants respectfully request that Examiner' rejection of claims 29, 31-42, 52, and 56 under 35 U.S.C. § 112, first paragraph be reversed.

2. Claim Rejections - 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 29-42, 52, and 56 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant [sic] regards as the invention. The Applicants respectfully submit that the Examiner has erred.

Rejections of this type are appropriate only where applicant has stated, somewhere other than in the application as filed, that the invention is something different from what is defined by the claims. In other words, the invention set forth in the claims must be presumed, in the absence of evidence to the contrary, to be that which applicants regard as their invention. *In re Moore,* 439 F.2d 1232, 169 USPQ 236 (CCPA 1971).

The Examiner provides no evidence that applicant has stated, somewhere other than in the application as filed, that the invention is something different from what is defined by the claims.

Further, MPEP 2173 states quite clearly that the Applicants may be their own lexicographers, so long as the scope of the claims is clear so the public is informed of what

would constitute infringement. MPEP 2173.02 goes on to state that "Examiners are encouraged to suggest claim language to the applicants to improve the clarity or precision of the language used, but should not reject claims or insist on their own preferences if other modes of expression selected by the applicant satisfy the statutory requirement."

When determining whether or not a claim meets the statutory requirement for definiteness the claim must not be analyzed in a vacuum. The test for definiteness under 35 U.S.C. § 112, second paragraph is whether, "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1 USPQ2d 1081 (Fed. Cir. 1986).

The Applicants respectfully submit that one of ordinary skill in the art would be informed of what is claimed when the claims are read in light of the specification.

The Applicants respectfully submit that the Examiner has misconstrued Claim 29. In the following subsections, the Applicants present additional arguments and evidence in support of their position that Claim 29 does particularly point out and distinctly claim subject matter which they regard as the invention. Applicants respectfully request that Examiner' rejection of claims 29-42, 52, and 56 under 35 U.S.C. § 112, second paragraph be reversed.

A. The phrase "highly purified"

The Examiner has rejected claims 29-42, 52, and 56 on the grounds that the phrase "highly purified" in the preamble of Claim 1 [sic., should be Claim 29] is "relative and subjective." See Office Action of February 6, 2004, p. 3. The Applicants respectfully submit that the phrase "highly purified" is well defined in the specification on, inter alia, p. 4, lines 20-28. A key element in this definition is the improved biocompatibility of alginates recited in

Claim 52 and prepared according to the process recited in Claim 29. This improved biocompatibility, in turn, is supported by the data presented in the specification on pp. 12 - 25 ("Examples of Performance"). In particular, please see p. 16, ln. 22 - p. 17, ln. 9. The purity of alginates recited in Claim 52, made using a process recited in Claim 29, is contrasted with that of alginates resulting from other processes at, among others, p. 4, lines 30 - 37. See also p. 3, lines 8-11 and p. 4, lines 6-13. See also Appendix B, p. 5, ln. 15 - p. 6, ln. 9.

It is therefore respectfully submitted that one of ordinary skill in the art would understand the phrase "highly purified" as used in Claim 29, and would therefore be informed of the scope of Claim 29.

B. The term "complex forming agent"

The Examiner has rejected claims 29-42, 52, and 56 on the grounds that the phrase "complex forming agent" is "uncertain as to meaning and scope." See Office Action of February 6, 2004, p. 3. The Applicants respectfully submit that the Examiner's rejection under 35 U.S.C. § 112, second paragraph concerning the phrase "complex forming agent" is in error. As explained and supported in Section 1 and citations therein, above, the term "complex forming agent" and the manner in which these substances act are known and well-defined to one of ordinary skill in the art. Please see Appendix D, Definitions 1-6. Specifically, please see Definition 1, "chelate", for an explanation of the manner of action. Further, as stated above, the function of the complex forming agent in the process recited in claim 29 would be very clear to one of ordinary skill in the art when the claim is read in light of the specification, as it should be.

It is therefore respectfully submitted that one of ordinary skill in the art would understand the term "complex forming agent" as used in Claim 29 and all claims depending therefrom, and

would therefore be informed of the scope of claim 29.

C. The phrase "raw algae material"

The Examiner has rejected claims 29-42, 52, and 56 on the grounds that the phrase "raw algae material" is "uncertain as to meaning and scope." See Office Action of February 6, 2004, p. 3. The Applicants respectfully submit that the Examiner's rejection under 35 U.S.C. § 112, second paragraph concerning the phrase "raw algae material" is in error. It is respectfully submitted that one of ordinary skill in the art will recognize that the terms "raw algae material" and "highly-purified alginate composition" as used in amended Claim 29 represent source material and end product respectively. One of ordinary skill in the art will also recognize that "raw algae material" includes both "commercially available raw alginate" and algae obtained in its natural state.

Support for the definition of "raw algae material" is to be found in the specification of the instant application. Beginning on Page 4, the Applicants make a clear distinction between, on one hand, "highly purified alginate," the end-product recited in Claim 52, and the result of the process recited in Claim 29 (p. 4, lines 20-28), and, on the other hand, "commercially available raw alginate" (p. 4, lines 30-37). The latter is described as "blends or mixtures of different algae materials, with the inclusion of animal or other foreign materials [which] therefore cannot in principle provide highly-purified alginate." At the same time, the Applicants elsewhere describe the source material as "clean, fresh algae material or dried algae material" (p.4, lns. 36-37) and material acquired "at the place of algae harvesting" (p. 5, lns. 37-38).

Claim 29 recites a process, which must be considered as a whole. The step of obtaining alginate in dissolved form is only one step in the process. One of ordinary skill in the art will

further recognize that a crucial distinction between the end product and all of these source materials is that the end product alone possesses high biocompatibility. Both types of source material are thus clearly delineated and contrasted with the end product, "highly purified alginate."

Further, please see Appendix B, p. 5, ln. 19 - p. 6, ln. 9; p. 8, ln. 17-22. See also Appendix C, p. 104, right column, second paragraph.

A crucial attribute of the highly purified end product is high biocompatibility (spec., p. 4, ln. 20-28), which is well established in the examples of the specification, pp. 12 - 25. Biocompatibility is a crucial distinction of the end product over all the source materials. "Alginates according to the invention are biocompatible, by contrast with conventional alginate extracts . . . " (p. 11, lns. 6-9.) One of ordinary skill in the art will recognize that "conventional alginate extracts" includes the previously mentioned "commercially available raw alginate." Direct comparison of the biocompatibilities of "Raw alginate" and of the materials recited in Claims 29 and 52, can be seen in the chart at p. 16, lines 25-36.

It is therefore respectfully submitted that one of ordinary skill in the art would understand the phrase "raw algae material" as used in the present Claims.

D. Formalities

In this sub-section, the Applicants respond to those grounds for rejection in the Office Action mailed February 6, 2004, which have not been addressed in the preceding sub-sections A-C. These are mainly of a formal nature and are presented on pages 3 and 4 of the Office Action, beginning with the 5th paragraph on p. 3.

The Examiner rejected claim 30-32 as not having clear antecedent basis for the terms "the extraction" and "the extracting." Upon remand to the Examiner from the Board, the Applicants will amend dependent Claims 30-32 to provide proper antecedent basis for all terms, including complex forming agent. References to the word "extracting", deleted from Claim 29, will be replaced by references to the word "treating" in Claim 29 as it now stands.

The Examiner rejected claim 31, stating that the term "soda solution" is uncertain. The Applicants respectfully submit that the term "soda solution" in Claim 31 and in the specification is definite to one of ordinary skill in the art, meaning a solution containing sodium, added, for example, in the form of Na_2CO_3 (Spec., p. 7, ln. 24; p. 13, lns. 17, 25). If the Board disagrees, the Applicants will propose an amendment to Claim 35 upon remand to the Examiner.

The Examiner rejected claim 34, stating that the term "on the basis of" is uncertain as to meaning and scope. The Applicants respectfully submit that the phrase "on the basis of" in line 2 of Claim 34, while perhaps not the ideal translation from the German original, will be understood in its context by one of ordinary skill in the art. If the Board disagrees, the Applicants will propose an amendment to Claim 34 upon remand to the Examiner.

The Examiner rejected claim 35, stating that the term "deep filters" is uncertain as to meaning and scope. The Applicants respectfully submit that the phrase "deep filters" in Claim 35, while perhaps not the ideal translation from the German original, will be understood in context from the specification by one of ordinary skill in the art as referring to ultrafiltration, or the use of filters having very small pore sizes (spec., p. 14, lns. 6-9). If the Board disagrees, the Applicants will propose an amendment to Claim 35 upon remand to the Examiner.

The Examiner rejected claim 40, stating that the term "fresh algae material" is uncertain. The Applicants respectfully submit that the meanings and scope of "fresh algae material" in

Claim 40 is definite to one of ordinary skill in the art. It is well defined in the specification (p. 4, lns. 36-37) and within Claim 40 itself. See also the several discussions involving the word "raw", in sub-section C, above, and subsection E, below.

The Examiner rejected claim 41, stating that the terms "organ" and "tissue" parts need to be differentiated from "algae parts." The Examiner further stated that the phrase "specific stages of the development cycle" is unclear as to meaning and scope. The Applicants respectfully submit that the difference between "organ" or "tissue" parts and "algae parts" in Claim 41 is definite to one of ordinary skill in the art. These are well-established biological terms. Cells, the basic unit of living things, may be organized into tissues, while tissues may in turn be organized into organs. At the same time, some cells, or groups of cells, may not be readily associated with a tissue or organ. Similarly, it is known to one of ordinary skill that all living things, including algae, have development cycles with specific, identifiable stages. The phrase "specific stages of the development cycle of algae", in the context of Claim 41 and the specification, is definite to one of ordinary skill.

E. The Applicants' response to the Examiner's "Response to Arguments"

The Applicants respectfully submit that the Examiner's statements under the heading "Response to Arguments" on p. 5 of the Office Action mailed on February 6, 2004, concerning the terms "raw" and "raw algae material", are in error. The Applicants offer the previous subsections in this Section 2 in support of their position, in particular, subsection 2C above. It is respectfully submitted that one of ordinary skill in the art will recognize that the terms "raw algae material" and "highly-purified alginate composition" as used in amended Claim 29 represent source material and end product respectively. One of ordinary skill in the art will also recognize

that "raw algae material" includes both "commercially available raw alginate" and algae obtained in its natural state. One of ordinary skill in the art will further recognize that a crucial distinction between the end product and all of these source materials is that the end product alone possesses high biocompatibility.

3. Claim Rejections - 35 U.S.C. § 103

The Examiner rejected claims 29-42, 52, and 56 under 35 U.S.C. § 103(a) as being unpatentable over Klöck, et. al. (AP) ("Klöck") *(Appl. Microbiol. Biotechnology vol. 40, 1994, pages 638 ff.)* in view of Nevins, et. al., (US 4,954,447), and Yeh (US 5,489,674), and if necessary in view of Zimmermann, et. al. (DE 42 04 012 A1). The Applicants respectfully request that the Board reverse this rejection.

In order to establish a prima facie case of obviousness three basic criteria must be met. First, there must be some suggestion or motivation in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference(s) must teach all of the claim limitations. "The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in the applicant's disclosure." *In re Vaeck*, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

Further, in determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. See, e.g. *In re Hirao*, 535 F.2d 67, 190 USPQ 15 (CCPA 1976).

The Applicants respectfully submit that the Examiner has erred in rejecting claims 29-42, 52, and 56 under 35 U.S.C. § 103(a) as being unpatentable over Klöck, et. al. (AP) ("Klöck") *(Appl. Microbiol. Biotechnology vol. 40, 1994, pages 638 ff.)* in view of Nevins, et. al., (US 4,954,447), and Yeh (US 5,489,674), and if necessary in view of Zimmermann, et. al. (DE 42 04 012 A1). As a preliminary matter, it is submitted that taken as a whole, the combination of Klöck, Nevins, Yeh and Zimmerman does not disclose each and every element of claim 29. Further, even if the combination of Klöck, Nevins, Yeh and Zimmerman were to disclose each element of claim 29, it is submitted that the Examiner has not succeeded in demonstrating motivation to modify or combine references as suggested, and has also has not provided evidence supporting a reasonable expectation of success in producing, using the referenced inventions, an alginate with as high a purity and biocompatibility as that recited in present Claims 29 and 52.

A. The Combination of Klöck, Nevins, Yeh and Zimmer does not Teach Every Element of Claim 29.

Applicants respectfully submit that Klöck teaches a process fundamentally different from that of present Claim 29 and provides no motivation for modification or combination with the other references cited by the Examiner. The differences can be seen in the following table, summarizing both processes (repetitions not included):

Klöck (p. 638, ff, "Procedure for alginate purification")	Claim 29
Preparing Ba^{2+} alginate "gels" or "beads" by "polymerization", i.e. adding alginate solution to a $BaCl_2$ solution.	a) treating raw algae material with a complex forming agent creating a liquid comprising dissolved alginate and solid matter;
"Supernatant removed using a metal sieve", beads retained.	b) filtering said liquid to produce a filtrate, said filtrate being a solution comprising dissolved alginate;
Treatment of alginate beads with acetic acid and sodium citrate. Ethanol extraction of beads.	c) precipitating said alginate out of said solution; (limited to precipitation with ethanol in Claim 36.)
Dissolving beads in strongly alkaline, EDTA-containing solutions	d) collecting and dewatering the precipitated alginate
Dialysis to remove Ba^{2+} ions and reagents	
Precipitation of alginate by adding ethanol	

Please see also Appendix B, Section 2, pp. 5-8.

Claims 29 and 52 are patentably distinct from the invention of the Klöck reference. Claim 29 recites a process, and must be considered *as a whole*. The process recited in claim 29 is a process for obtaining a highly purified alginate composition, which comprises treating raw algae material with a complex forming agent, which creates a liquid comprising dissolved alginate and solid material. The liquid is filtered to remove the solid material and provide a

filtrate solution comprising dissolved alginate. The dissolved alginate is precipitated out of solution, then collected and dewatered. These steps are repeated one or more times.

It is Examiner's contention that since the process disclosed by Klöck includes as a final step, a precipitation of an alginate material that Klöck necessarily discloses the same process as that recited in claim 29 of the instant application. But, as stated above, the claimed process must be considered as a whole, as must the process disclosed by Klöck.

Both the process recited in claim 29 of the instant application and the process disclosed by Klöck start with a raw alginate material, which may be a commercial alginate material. See Klöck at page 640, first column. See Example 1 of the instant Application. However, the purification means employed by the process of claim 29 and Klöck differ radically. In the process of Klöck a solid complex of alginate with barium in the form of beads is formed. These barium alginate beads are subsequently purified by extraction with acid and alcohol. The solid beads are subsequently treated with a highly alkaline solution of EDTA, after the purification steps, to break up the barium complex and dissolve the alginate. The recovered alginate is precipitated by the addition of alcohol after dialysis to remove barium ions.

In contrast, claim 29 recites a process wherein the alginate is dissolved by treatment with a complex forming agent and subsequently separated from solid impurities by filtration. The process of claim 29 does not require the formation of solid barium alginate beads to obtain a purified alginate.

The Examiner hypothesizes that the barium beads of Klöck may be considered as the raw material for the process recited in claim 29. See Office Action of February 6, 2004, p. 7. The Examiner further posits that the treatment of the barium beads with highly alkaline EDTA, and subsequent precipitation with ethanol, as taught in Klöck may provide further purification. Id. at

p. 8. The Examiner also contends that claim 29 does not exclude the steps of extracting barium alginate beads with acid and alcohol prior to dissolution by treatment with a complex forming agent. However, there are several basic flaws in the Examiners reasoning.

First, the Examiner tries to find a commonality between the process of Klöck and the process recited in claim 29 by stating that the commercial alginate used as a raw material in the process of Klöck is the same as the raw material "required" by claim 29. See Office Action of February 6, 2004, pp. 6-7. However, starting from this basis, it is clear that Klöck and the process of claim 29 purify the raw algae material in fundamentally different ways. Klöck forms barium alginate beads from the raw algae material, which are extracted with acid and alcohol. The process of claim 29 dissolves the raw algae material by treatment with a complex forming agent, followed by filtration and precipitation. On this basis alone, Klöck does not disclose every element of claim 29.

Having started his analysis by linking Klöck and the process of claim 29 on the basis of a common starting material, the Examiner then goes on to state in the same paragraph that the barium beads of Klöck can be considered the raw material for the process recited in claim 29. However, it is clear that the Examiner recognizes the difference between the raw algae material that both Klöck and the process of claim 29 use as a raw material and the purified barium alginate beads that result from the purification step in Klöck. The barium beads of Klöck are not raw algae material with respect to the Klöck process; they are its purified product.

Further, the Examiner's contention that the dissolution of the barium beads using highly alkaline EDTA, and the subsequent precipitation of the alginate using ethanol may provide additional purification is nowhere supported in the disclosure of Klöck. Only the instant application discloses that dissolution of alginate with a complex forming agent followed by

filtration and precipitation with ethanol provides purified alginate. Klöck simply does not disclose this key element of the process recited in claim 29.

The Examiner's contention that the process recited in claim 29 does not exclude the steps recited in Klöck does not save his argument. As conceded by the Examiner, both Klöck and the process recited in claim 29 start with raw algae material. The fact that the process of claim 29 dissolves the raw algae material by treatment with a complex forming agent by definition excludes forming barium alginate beads. As stated above, the purified barium alginate beads are not raw algae material with respect to the Klöck process.

Looking to Nevins, Yeh and Zimmerman, none of these references cure this fundamental deficiency of Klöck. The Applicants therefore respectfully submit that the combination of Klöck, Nevins, Yeh and Zimmerman does not disclose each and every element of claim 29.

The Examiner's statement that ". . . sodium alginate is normally formed by treating algae with alkali in the absence of EDTA. . . " (Supplementary Action page 3, lines 11 - 12), is inapposite since the barium alginate beads that are treated with alkaline EDTA are already purified.

Further, the Examiner likewise provides no evidence for the statement that ". . . when adding sodium EDTA as set forth in the declaration (third page), the EDTA may be functioning only to supply sodium ions to form sodium alginate that is soluble" (Supplementary Action, page 3, lines 8-10). The Applicants respectfully submit that the Examiner has misconstrued the process of Claim 29, and that the complex forming agent effectively separates the alginate from other impurities, in addition to multivalent cations. The applicants offer the evidence in this section and the previous sections of this brief in support of this.

B. There is No Motivation to Combine Klöck, Nevins, Yeh, and Zimmerman as Suggested by the Examiner.

Even if Klöck were to disclose each and every element of claim 29, the Klöck reference provides no motivation to modify its teachings as the Examiner suggests. Further none of Nevins, Yeh, and Zimmerman, provides motivation to modify or combine references to lead to the invention of Claims 29 and 52. Claim 29 and all Claims depending therefrom except Claim 52 recite a process, which must be considered *as a whole*. Claim 52 recites a composition made with the process, which differs significantly from that of these references in purity and biocompatibility and is not rendered obvious by the references separately or in combination.

As stated above, considering the process of Klöck and the process recited in claim 29 as a whole, they produce a purified alginate from raw algae material in fundamentally different ways. In order to modify the process of Klöck to arrive at the process recited in claim 29, one would be required to remove completely from Klöck the steps of forming barium alginate beads and extracting the barium alginate beads to purify them. This modification would completely vitiate the core teachings of Klöck. If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the reference are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

Modifying the process of Klöck to remove the step of forming barium alginate beads, and replacing that step with the step of dissolving the raw alginate material by treatment with a complex forming agent would change the principle of operation of the Klöck process. Therefore, there can be no motivation to modify Klöck as suggested by the Examiner.

C. There is No Reasonable Expectation of Success in Modifying Klöck.

The Applicants respectfully submit that the Examiner has provided no evidence that one of ordinary skill in the art would have a reasonable expectation of success in modifying or combining the Klöck reference to produce an alginate composition with purity and biocompatibility equivalent to that of Claims 29 and 52.

The prior art can be modified or combined to reject claims as prima facie obvious as long as there is a reasonable expectation of success. *In re Merck & Co., Inc.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). "The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in the applicant's disclosure." *In re Vaeck*, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).
In Klöck the alginates are first polymerized (Appendix B, p. 5, lns 4-5; p. 7, ln. 21 - p. 8, ln. 2) using Ba^{2+} ions, then purified with acids at relatively high temperatures. It would be recognized by one of ordinary skill in the art that the alginate so treated will be physically and chemically modified with respect to the alginate composition recited in present Claim 52 and prepared as recited in Claim 29. See Appendix B, p. 8, lns. 3-22. The process of the references continues with further purification using alcohol, and finally dissolution of the barium alginate complex by treatment with highly alkaline EDTA, after the purification steps. This process cannot be used to extract alginate directly from naturally occurring algae material.

By contrast, present Claim 29 recites a process in which the alginate is purified in a dissolved state in the beginning. This recited process can be used to extract alginate directly from naturally-occurring algae material. No acid or heat is used. It would be recognized by one of ordinary skill in the art that this process is distinct from that in the references in that it involves different steps and results in a chemically and physically distinct final alginate.

In order to modify Klöck to conform to the process recited in claim 29, one would have to remove the steps of (1) forming a barium alginate beads, (2) incubating the barium alginate beads with an acid, and (3) extracting the barium alginate beads with an alcohol. In short, one would have to remove the steps of Klöck that supposedly result in the purification of the alginate.

As recited in claim 29, the starting material is a raw algae material. Treatment with a complex forming agent causes some of the alginate to dissolve, and impurities remain in the solid matter (See current specification, p. 7, lns. 21-29; p. 4, ln. 30 - p. 5, ln. 16; p. 3, ln. 36 - p. 4, ln. 4; p. 1, lns. 10-28, p. 5, ln. 18 - p. 7, ln. 13). This is antithetical to the prior art, where the contaminants are dissolved to leave a solid alginate complex.

One of ordinary skill in the art could not have had any reasonable expectation of success in purifying algae material using a complex forming agent based on anything in the Zimmerman and Klöck references. Both of these references relate to the acid washing/chemical treatment of solid alginate beads to degrade the contaminants into the then discarded solution. In these references, commercial alginate is first precipitated into solid beads with barium chloride. To purify this solid alginate, it is incubated with a strong acid solution at a high temperature. According to the reference, the acids dissolve the contaminants, purifying the alginate.

For example, the Klöck reference teaches that "the contaminants were eluted by treatment of [the] Ba^{2+} [alginate] beads using different reagents followed by ethanol extraction." (Klöck, p. 640, first column, lns. 16-25). In particular, the alginate beads are suspended in "4.5 [liters] of 1 N acetic acid" and incubated for 14 hours to remove impurities. (Id., lns. 26-34). These alginate beads are not dissolved until the barium is removed, and EDTA is used only in

this final recovery step, to dissolve and recover the alginate from the by then acid purified, solid barium alginate. (Id., lns. 45-52.).

The Examiner has not provided any rationale for one of ordinary skill in the art to have a reasonable expectation of success in purifying raw alginate material by removing the purification steps taught by Klöck. Further, none of Nevins, Yeh or Zimmerman provides the reasonable expectation of success that is absent from Klöck.

D. The Applicants' response to the Examiner's "Response to Arguments"

The Applicants respectfully submit that the Examiner's statements under the heading "Response to Arguments" beginning on p. 8 of the Office Action mailed on 02/06/2004, are in error. The Applicants offer the previous subsections A and B of Section 3 in support of their position. Please see also Subsection 2C concerning the phrase "raw algae material", above.

To repeat, the process of Claim 29 and the product of Claim 52 are patentably distinct from prior inventions, including that of Klöck et. al.. In Klöck, the alginates are first put into the form of a barium complex, then purified with acids at relatively high temperatures. It will be recognized by one of ordinary skill in the art that the alginate so treated will be physically and chemically modified with respect to alginate composition recited in present Claim 52 and prepared as recited in Claim 29. See Appendix B, p. 8, ln. 3-22. The process of the references continues with further purification using alcohol, and finally dissolved. This process cannot be used to extract alginate directly from naturally occurring algae material.

The superior purity and biocompatibility of the product of Claim 52 are demonstrated by the data in the specification, starting on p. 12 (Examples of Performance). See, in particular, p. 16, ln. 23 - p. 17, ln. 3. The Examiner has not considered the subject matter of Claims 29 and 52

as a whole. The Examiner has not demonstrated that modification of or combining with the Klöck reference will yield a reasonable expectation of success.

Furthermore, the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified. Therefore the teachings of the reference are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). The Applicants respectfully submit that the modification of the Klöck reference implied in the Examiner's rejections cannot be carried out without changing its principle of operation.

The modification of the Klöck reference implied in the Examiner's rejections amounts to a process comprising, in each repetition, the steps of taking naturally occurring algae containing multivalent cations (Appendix B, p. 6, lns. 3-4) creating a sodium (monovalent) - alginate material ("commercial alginate"), adding barium ions, forming thereby once again a barium-alginate solid (multivalent), then using a complex forming agent to resubstitute monovalent ions for the barium. The Examiner submits that this renders obvious steps a) through d) of Claim 29. He offers, however, no evidence that one of ordinary skill in the art would reasonably expect this would yield an alginate of superior purity. The Klöck reference, therefore provides no motivation to modify, and is therefore not a valid reference for rejection under 35 U.S.C. § 103.

E. Conclusion

In sum, the Klöck reference teaches that alginate purity is improved when contaminants are dissolved in acid while the alginate remains solid. In contrast, Claim 29 contradicts this teaching: the alginate is dissolved while the contaminants remain in the solid and are removed by filtration. On this basis alone, the examiner has failed to establish a case of *prima facie*

obviousness since the combination of Klöck with Nevins, Yeh and Zimmerman does not teach each and every element of claim 29. Further, there is nothing in the references to motivate one of ordinary skill in the art to try the process of the present Claim 29. In fact, because modifying Klöck would change its principle of operation, there can be no motivation to modify Klöck as suggested by the Examiner.

The Applicants further respectfully submit that the Examiner has provided no evidence that modifying the Klöck reference or combining it with those of Nevins, Yeh, and if necessary Zimmermann, will have a reasonable expectation of success in producing an alginate composition with purity and biocompatibility equivalent to that of Claims 29 and 52. In addition, the Applicants have offered evidence that there is no such reasonable expectation.

Therefore, the Applicants respectfully submit that the Examiner erred in rejecting claims 29-42, 52, and 56 under 35 U.S.C. § 103(a) and request that the Board reverse the rejection.

Conclusion

The Applicants therefore respectfully request that the Board reverse the rejection of claims 29-42, 52, and 56 under 35 U.S.C. §§ 112, first and second paragraph and 103(a).

Respectfully submitted,

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Date: September 15, 2004

APPENDIX A

A process for obtaining a highly-purified alginate composition, the process comprising the steps of:

a) treating raw algae material with a complex forming agent creating a liquid comprising dissolved alginate and solid matter;

b) filtering said liquid to produce a filtrate, said filtrate being a solution comprising dissolved alginate;

c) precipitating said alginate out of said solution;

d) collecting and dewatering the precipitated alginate; and

e) repeating the steps a) to d) at least once.

30. A process according to Claim 29, wherein ethylene diamine tetra acetic acid is used as a complex forming agent for the extraction.

31. A process according to Claim 29, wherein the extracting takes place in a soda solution.

32. A process according to Claim 30, wherein activated carbon is added for the extraction of the solution.

33. A process according to Claim 29, wherein, before the filtering of the solution, sedimentation of cell constituents and particles from the solution is carried out with a porous binding agent.

34. A process according to Claim 33, wherein the sedimentation takes place with a porous granulate on the basis of diatomaceous earth, cellulose, or recycling materials from regenerated raw materials.

35. A process according to Claim 29, wherein the filtering takes place with deep filters, and the solution is subjected to more than one filtration, and the pore size of the filters used decreases for successive filtrations.

36. A process according to Claim 29, wherein the precipitation of the alginate takes place in a solution containing ethanol.

37. A process according to Claim 36, wherein the ethanol content is selected in the range from about 10% to about 50%.

38. A process according to Claim 29, wherein the collecting of the precipitated alginate is effected by foaming out of the solution, by decanting from the solution, or by stirring the solution with a stirring and collecting device.

39. A process according to Claim 29, wherein the dewatering of the alginate takes place at room temperature.

40. A process according to Claim 29, wherein the algae material used in the process is fresh algae material occurring in nature, fresh algae material cultivated in a bioreactor or tank system, or algae material from fusioned or regenerated algae cells.

41. A process according to Claim 29, wherein the algae material used in the process is specific organ or tissue parts of algae or algae parts, or specific organ or tissue parts of algae or algae parts from specific stages of the development cycle of algae.

42. A process according to Claim 29, wherein the algae material used in the process is an alginate-producing fresh-water or salt-water algae.

52. An alginate composition, manufactured by the process according to Claim 29, comprising a mixed polymer of mannuronic acid and guluronic acid, in which the ratio of mannuronic acid to guluronic acid in the mixed polymer is in the range from about 0.1 to about 9, and the mean molecular weight of the mixed polymer is greater than about 250 kD.

56. A process according to Claim 29 wherein the algae material used in the process is brown algae.

APPENDIX B

Attorney's Docket No. 113737.6 ***Patent***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zimmerman, et. al.			
Serial No.:	09/762,850	Group No.:	1651
Filed:	April 13, 2001	Examiner:	David M. Naff
For:	Method for Producing Ultra-Pure Alginates		

SUPPLEMENTAL REPLY UNDER 37 C.F.R. § 1.111

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Advisory Action mailed on September 2, 2003, which followed Applicant's response to the Office Action mailed on May 6, 2003, in the subject application, Applicants respectfully submit the following Declaration.

Applicants respectfully request that this Declaration be appended to the Request for Continued Examination, which was submitted on November 6, 2003 in the subject application.

AUTHORIZATION

Applicants believe that no fees or extension of time are required for this submission. However, in the event that an extension of rune is required, Applicants hereby submit a petition for such extension of time as may be necessary to make this response timely. The Commissioner is hereby authorized to charge any necessary fees to deposit account No. 502194. A duplicate of this Authorization is enclosed.

Respectfully Submitted,

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Date: January 29, 2004

AUTHORIZATION

Applicants believe that no fees or extension of time are required for this submission. However, in the event that an extension of time is required, Applicants hereby submit a petition for such extension of time as may be necessary to make this response timely. The Commissioner is hereby authorized to charge any necessary fees to deposit account No. 502194. A duplicate of this Authorization is enclosed.

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Date: January 29, 2004

Attorney's Docket No. 49865.2 ***Patent***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zimmerman, et. al.			
Serial No.:	09/762,850	Group No.:	1651
Filed:	April 13, 2001	Examiner:	David M. Naff
For:	Method for Producing Ultra-Pure Alginates		

DECLARATION OF Dr. Frank Thürmer UNDER 37 C.F.R. § 1.132

I, Dr. Frank Thürmer, am a researcher. I received my PhD in natural science from the Bayerischen Julius-Maximilians-Universität Würzburg in 1998.

I am the head of production at CellMed AG and head the encapsulation and biopolymer team. I have extensive experience in the field of biotechnological, biomedical and biophysical research. Prior to the current employment I was 3 years the responsible project leader of the team "immobilization artificial organ replacement" at the Department of Biotechnology at the University of Würzburg. I headed several major grant projects and I am the author of about 15 publications, concerning among other thinks the properties of alginate.

I am familiar with the process and product that is disclosed and claimed in the above-captioned U.S. Patent application, and I consider myself skilled in the art pertaining to this application. I declare the following in support of the Request for Continued Examination submitted on November 6, 2003, in the above-captioned application.

1. The Nature of Alginate Salts

Alginates are soluble as salts only if the corresponding cation is monovalent (e.g. Na-or K- alginate). Alginates are not soluble as acids or as salts of multivalent cations (e.g. Ba-or Ca-alginates). The reason for this lack of solubility is the fact that with multivalent cations the alginate polymer chains are connected with ionic bonds. Further, alginates have a higher affinity with multivalent cations than with monovalent cations.

Multivalent cation salts of alginates can be dissolved by adding a complex-forming agent such as EDTA to remove the multivalent cations, while supplying surplus monovalent cations to form a new soluble salt with the alginate. An adequate concentrated EDTA solution for example will dissolve Ca- and Ba-alginate, whereas for example a citric acid-solution will only dissolve Ca-alginate.

2. Differences between the present invention and the prior art

A. Precipitation

According to DE 42 04 012 (and Klöck et al. 1994), the starting material is dissolved Na alginate (see DE 42 04 012, column 3, line 47, column 4, line 18, Klöck et al., page 640, left column, § 3, 1st sentence). Addition of BaCl in these prior processes yielded Ba-alginate particles, which are insoluble (see above) and therefore can be separated from the solution. The aim of this prior art separation was an attempt to separate the alginate from mitogenic substances. Because the earlier results with the prior technique still contain disadvantages (see examples in the present application), the present invention has been developed.

In contrast to the prior techniques, the invention described in the present application teaches in a first step dissolving the alginate from the plant material, in particular from the cellwalls. In the plants (in the raw material), the alginates are bound with multivalent cations. Therefore, a complex forming agent is used, which has a higher affinity to the multivalent cations than does the alginate. An example of such an agent is EDTA. As the result of this step an alginate can be dissolved by removing the multivalent cations. The alginate is thus contained in the solution in a dissolved condition, and it can be separated from the solid plant components by filtration in the next step, the alginate being dissolved in the filtrate.

To make it quite clear, it is not the alginate which is complexed by the complex forming agent. In solution EDTA is anionic, as is the alginate, and for this reason an EDTA/alginate complex cannot be formed. The complex forming agent complexes only differently charged compounds, i.e. cations. At the same time, monovalent cations become bound to the alginate, which thus becomes soluble. The monovalent cations are either contained in the plant material (cell walls) in a sufficient amount (note: The algae grow in salt water) or may be added, e.g. as in example l of the specification in the form of sodium carbonate, or, as in example 2 of the specification, in the form of sodium EDTA (Ethylenenediamine tetraacetic acid tetrasodium salt) as complex forming agent. The suspension mentioned in example 1 results from using dry raw algae material for extraction, i.e. complete plant material with all soluble and insoluble components. During the extraction process the alginate goes into solution (because multivalent cations are bound to the complex forming agent and are exchanged for monovalent cations binding to

the alginate) whereas the insoluble plant material such as cell walls etc. form a suspension in the alginate solution.

In commercial alginate the multivalent cations already have been exchanged for monovalent cations by extraction, and this is the reason why it is soluble in the absence of EDTA.

In DE 42 04 012, Barium is used for precipitating alginate, while in the present invention the complex forming agent is used for dissolving the alginate into the solution by removing multivalent cations. According to the present invention, the complex forming agent does not form a complex with the alginates, which is clear from the further steps of the process.

B. Purification

The present invention is new over the references (DE 42 04 012 and Klöck et at.) concerning purification of the alginate. According to the prior techniques, the material precipitated with Ba^{++} is purified by subsequent steps. On the other hand, purification with the present invention is obtained just from filtration and precipitating with alcohol. According to the present invention, the alginate is purified by precipitating it out of the solution that was previously formed by adding the complex-forming agent. This precipitation is done by adding e.g. ethanol to the solution. The addition of ethanol to the aqueous solution results in the fact that the dissolved alginate cannot be kept in the dissolved condition any longer. The alginate is thus precipitated as a salt. In this sense, this precipitation step in the present invention is a real precipitation. By contrast, the reaction

of alginate with Ba according to the prior techniques represents a cross-linking of the polymer chains. This cross-linking yields big molecules which are no longer soluble.

The inventive process distinguishes over the Zimmermann and Klöck references since the processes of these references start from dissolved commercial sodium or potassium alginate which is precipitated by barium or other multivalent cations to form an insoluble crosslinked complex. This complex is treated with acids at high temperature for purification. A disadvantage of this process is that the alginate is chemically and physically modified. The complex is then washed and treated with alcohol to dissolve alcohol-soluble contaminants. In a last step the water-insoluble alginate complex is dissolved with EDTA. By contrast, in the present invention, what is purified is an alginate extracted into solution, not a solid alginate. No acid or heat is used, and alcohol is used to precipitate the alginate from the solution. With the Zimmermann and Klöck process, already extracted alginate can be purified, but it is not possible to extract alginate from raw algae material. It should be noted that the present invention is not simply a process for dissolving alginate with EDTA; the EDTA extraction is just one step in a series of specific purification measures.

Commercially available alginates, are often prepared from mixtures of different algae species leading to a huge charge variability, concerning the physical and chemical properties of the alginate (Orive et al. 2003; page 104, right column, second paragraph). Even if all the impurities are removable according to the prior inventions, the physical properties will vary from charge to charge. The present invention has the advantage that standardized material from single species can be used, leading to a reproducible product.

I declare that the foregoing is true and correct, that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by free or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12.01.2004

Dr. Frank Thürmer

APPENDIX C

COMMENTARY

In cell encapsulation, transplanted cells are protected from immune rejection by an artificial, semipermeable membrane, potentially allowing transplantation (allo- or xenotransplantation) without the need for immunosuppression. Yet, despite some promising results in animal studies, the field has not lived up to expectations, and clinical products based on encapsulated cell technology continue to elude the scientific community. This commentary discusses the reasons for this, summarizes recent progress in the field and outlines what is needed to bring this technology closer to clinical application.

Cell encapsulation: Promise and progress

GORKA ORIVE[1], ROSA MARÍA HERNÁNDEZ[1], ALICIA R. GASCÓN[1], RICCARDO CALAFIORE[2], THOMAS M.S. CHANG[3], PAUL DE VOS[4], GONZALO HORTELANO[5], DAVID HUNKELER[6], IGOR LACÍK[7], A.M. JAMES SHAPIRO[8] & JOSÉ LUIS PEDRAZ[1]

In 1964, T.M.S. Chang[1] proposed the idea of using ultrathin polymer membrane microcapsules for the immunoprotection of transplanted cells and introduced the term 'artificial cells' to define the concept of bioencapsulation, which was successfully implemented 20 years later to immobilize xenograft islet cells. When implanted into rats, the microencapsulated islets corrected the diabetic state for several weeks[2]. Since then, there has been considerable progress toward understanding the biological and technological requirements for successful transplantation of encapsulated cells in experimental animal models, including rodents and non-human primates. Bioencapsulation has provided a range of promising therapeutic treatments for diabetes[3], hemophilia[4], cancer[5] and renal failure[6]. Additionally, the functional applicability of cell encapsulation in humans has also been reported in several clinical trials[7,8].

Despite considerable interest, however, the field has not lived up to expectations[9]. A lack of reproducibility, or uncertainty surrounding the reproducibility of prior studies has been a major problem. For example, in the treatment of diabetes, researchers have been plagued by the inability to reproduce one excellent trial in dogs undertaken by Metabolex of Hayward, California (http://www.metabolex.com), one important primate study on rhesus monkeys[3] and a single patient study[7]. To put it simply, despite the Edmonton protocol[10], in which islet transplantation in patients with type I diabetes resulted in insulin independence for a year, very few groups have isolated sufficiently good islets and developed a suitable biocompatible encapsulation material.

Indeed, the consensus is that microencapsulation still represents a sort of 'in-house' procedure, administered by a small number of laboratory groups who are reluctant, or who are unable because the technology is proprietary, to share complete information and protocols. Also retarding progress of this field is a lack of standardized technology, including optimized tissue and clinically proven materials for membrane manufacturing produced in reproducible batches. These limitations have bedeviled universities and startup companies alike. University-based laboratories have had the added difficulty of scaling up technologies to produce materials in sufficient quantities to permit duplicate studies. As a consequence, many essential research questions, such as the exact selection of membrane materials, their final properties, site of transplantation, cell source and choice of purification methods, remain unanswered.

Current challenges

Technological and biological limitations, as well as ethical, political and regulatory obstacles, must be overcome if the promise of cell encapsulation technology is to be realized. Some of the important considerations for consistent clinical success of cell encapsulation include a source of functional cells; a biocompatible, as well as mechanically and chemically stable, membrane of a suitable permeability cut-off value that provides immune protection to the implant; functional performance; biosafety; and long-term survival of the graft.

Lack of clinical-grade polymers. In the quest for a better microencapsulation design, many types of natural and synthetic polymers are being explored. A substantial challenge related to the biomaterials used in cell encapsulation has been the lack of clinical-grade polymers. Although its intrinsic properties make alginate the current encapsulation material of choice, batches of alginate need to be standardized to minimize endotoxin and protein content, both of which can affect biocompatibility. This requires standardized protocols to eliminate such impurities[11]. In this regard, an international task force should be instituted to set up a 'central alginate factory' that would prepare standardized prototypes for use by participating laboratories in their transplant studies. The European Community and Norway now support the formation of such dedicated centers.

Production of uniform capsules. Another challenge involves the production of uniform capsules with excellent repeatability and reproducibility both within and between batches. The adoption of automated machines for microencapsulation could result in improved reproducibility in terms of shape, size and morphology. As such, the recent development of an automated chemical reactor[12] may help to address this problem.

Use of polycations. The discovery of suitable immune-compatible polycations represents another principal area of study. After 20 years of research, the clinical application of polycations for microcapsule formulation remains controversial. Although some believe that poly-L-lysine (PLL) polycation has a low probability of success as a result of its poor biocompatibility[13], others have obtained promising *in vivo* results replacing PLL with poly-L-ornithine[14] or poly(methylene-co-guanidine) hydrochloride[15]. It may be that ultimately the simplest system of them all, the alginate bead with its non-uniform density, will be sufficient to provide immune protection in the case of allogeneic models (intraspecific human), whereas the development of microcapsule materials for xenogeneic models (interspecific

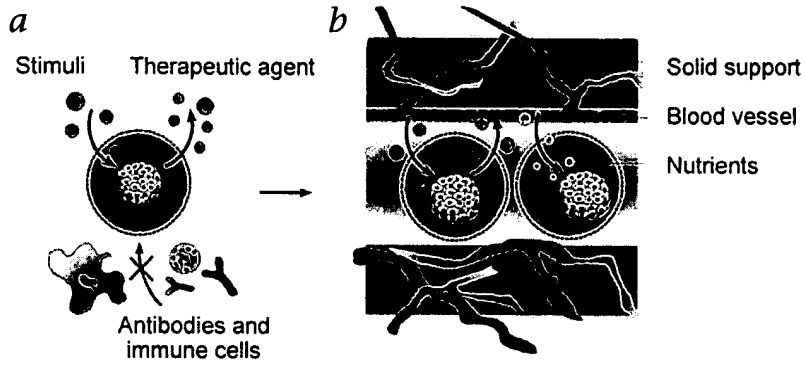

Fig. 1 Cell microencapsulation. *a*, Nutrients, oxygen and stimuli diffuse across the membrane, whereas antibodies and immune cells are excluded. *b*, Pre-vascularized solid support system to facilitate optimal nutrition of the enclosed cells.

nonhuman) will remain a challenge. This is because alginates are too porous to offer suitable immune protection to the implant.

Considerations before transplantation. Still other challenges involve the assessment of the exact dosage[10] and molecular-weight cutoff value, as well as the overall biocompatibility of the system. X-ray photoelectron spectroscopy and Fourier-transform infrared spectroscopy techniques could shed light on the latter, helping to identify the chemical groups causing bioincompatibility on the surface of a capsule and predicting the biosafety of the devices before implantation[16].

Selecting suitable cell types for immobilization. A number of issues should be carefully evaluated when selecting suitable cell types for immobilization. Indeed, encapsulation requires an appropriate source of functional cells. In this regard, the potential use of allogeneic versus xenogeneic cells has provoked important social and ethical debates[17]. The principal controversy surrounds the potential risk of inadvertent transfer to humans of animal viruses present in the xenotransplant, particularly the porcine endogenous retrovirus[18], and many forums have concluded that research should proceed with allotransplantation over xenotransplantation[19]. Regulatory issues and selective moratoriums[20–22] aside, xenogeneic graft pilot trials could benefit from the use of special multicompartmental microcapsules. These may embody, for example, anti-oxidizing, anti-apoptotic and β-cell pro-mitogenic factors that could prolong primary cell survival and provide functional competence[23].

Transplantation site. The choice of transplantation site is another important consideration. Here, it is necessary to weigh issues such as the safety and possibility of re-transplantation (peritoneal cavity, subcutaneous transplantation) against proximity to the circulation[24] (intrahepatic transplantation or membranes supporting vascularization). Still another pertinent issue is that permanent graft survival of encapsulated cells has never been reported. Some groups attribute these graft failures to the lack of direct vascularization of the enclosed cells, with consequent gradual tissue necrosis and death. To address this problem, pre-vascularized solid supports are being studied to improve the nutrition of the encapsulated cells[25] (Fig. 1). However, the question of vascularization remains open, because other groups have obtained promising results by transplanting capsules into the peritoneal cavity of large animals without direct contact with blood vessels[14].

Regulatory and ethical issues

With advances in the science of encapsulated cell therapies, regulatory authorities have been gradually adjusting their policies to accommodate these new therapeutic approaches. In the United States, for example, all islet transplant studies (and presumably all future encapsulation-type clinical studies) will be regulated by the US Food and Drug Administration (FDA) under an investigational new-device submission. For now, Europe will likely rely on FDA guidelines, because specific regulations in this field are presently lacking. Recently, the US Pharmacopeia and National Formulary included a new therapeutic category for cell-based products[26], which constitutes a significant step toward accepting this technology and encouraging clinical trials.

A major ethical concern surrounding the use of microencapsulated cells is to ensure that patients are treated with a technology that demonstrates a clearly proven biosafety based on standardized protocols and procedures. In this regard, it is important to avoid poorly conducted studies that put individuals at unnecessary risk and unfairly raise hopes and expectations. For example, the recent trial of pig islet xenotransplantation in children by Valdes' group at the National University of Mexico has sparked a fresh round of debate[27], because it is in direct contravention of the Helsinki Agreement on the ethical performance of clinical trials. Moreover, reports of this kind run the risk of hampering future progress of the entire field.

Recent progress

In recent years there have been some interesting research developments. For example, considerable effort has focused on the identification of alternative natural and synthetic materials for use in cell encapsulation. These efforts have resulted in the polyanionic material recently patented under the name Biodritin[28], the photopolymerizable poly(ethylene glycol) polymer to immobilize cell clusters (http://www.novocell.com, Irvine, California), and the genetically modified alginates with a highly controlled chemical structure[29]. Recently, microfabricated silicon membranes have been reported to shed light on the issue of permeability control[30]. This thin membrane possesses an extremely uniform pore size of only a few nanometers wide, providing strict control over the inward diffusion of immunoglobulins and, hence, greater protection against immunorejection of the transplanted cells.

In addition, genetic engineering has contributed to the development of modified cells that have superior cell viability and are therefore capable of providing an improved supply of therapeutic products. In one study, recombinant

mouse myoblasts enclosed in alginate-polylysine-alginate microcapsules, showed continuous expression of human Factor IX for at least 7 months *in vivo*[4]. In another study, hybridoma cells enclosed in cellulose sulfate capsules showed detectable levels of antibody in mouse serum for as long as 4 months after transplantation[31], indicating that this approach might be useful for antibody-based gene or cell therapy. In regard to cancer therapy, researchers used encapsulated genetically modified allogeneic cells expressing pro-drug-activating enzymes such as cytochrome P450 (ref. 32) as a possible treatment for inoperable pancreatic carcinoma. Moreover, promising anti-angiogenic results have been obtained with endostatin-transfected cells encapsulated in alginate in the treatment of malignant brain tumors[33,34]. Finally, another group has developed an approach to tumor suppression that involves genetically modified myoblast cells secreting interleukin-2 linked to the Fv region of a humanized antibody with affinity to tumors overexpressing the oncogene *ERBB2* (also known as *HER-2* or *NEU*)[35].

The gross insufficiency of suitable cadaveric and fetal cells could likely be circumvented through the use of stem cells. Once suitable sources of stem cells and appropriate means to control their differentiation become available, stem cells may constitute a universal cell line suitable for the large-scale manufacture of encapsulation devices. In any event, microencapsulation may be necessary for the immunoisolation of stem cells, in that recent studies have shown differentiated human embryonic stem cells to express high levels of major histocompatibility (MHC) class I proteins, which may cause them to be rejected on transplantation[36].

What the future holds

Cell microencapsulation is a technology with enormous clinical potential for the treatment of a wide range of diseases[37]. Yet many difficulties remain, some of which will certainly challenge our scientific ingenuity. The stepwise analysis of the essential obstacles, coupled with increased international collaboration, should move the technology forward in a careful and controlled way and bring it much closer to clinical reality. Some of the most convincing arguments for bioencapsulation are that it could eliminate the need for immunomodulatory protocols or immunosuppressive drugs and permit the long-term *de novo* delivery of therapeutic products in either a local or systemic manner[38].

Clinical trials of alginate microcapsules could begin soon. An application to initiate clinical trials of encapsulated human islets in non-immunosuppressed patients with type 1 diabetes, headed by Riccardo Calafiore and Paolo Brunetti of the University of Perugia, is currently pending at the Italian Ministry of Health. Clinical applications for other endocrine defects, such as pituitary dwarfism or thyroid and parathyroid disorders, are expected to follow.

Despite these promising developments, we believe that if cell encapsulation technology is to receive a full evaluation as a potential therapeutic alternative to overcome the limitations of whole-organ transplantation (such as, lack of suitable donors, need for immunosuppression, potential risks associated with major operation, cost), an international advisory committee should supervise the initiation of pilot clinical trials of microencapsulated cell allografts into carefully selected recipients. This paper represents a call for such a committee.

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APPENDIX D

Hawley's
Condensed Chemical Dictionary
Fourteenth Edition

Revised by
Richard J. Lewis, Sr.

JOHN WILEY & SONS, INC.

complexing agent. See ligand; chelate; ethylenediaminetetraacetic acid.

Def. 1

"Chel" *[Novartis].* TM for chelating agents based on polyaminocarboxyllic acids.
Use: To reduce the harmful effects of trace metals in the blood.

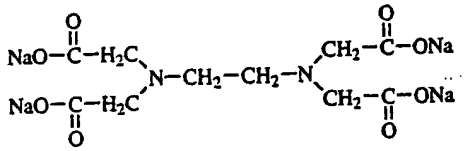
chelate. The type of coordination compound in which a central metal ion such as Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+} is attached by coordinate links to two or more nonmetal atoms in the same molecule, called ligands. Heterocyclic rings are formed with the central (metal) atom as part of each ring. Ligands offering two groups for attachment to the metal are termed *bidentate* (two-toothed); three groups, *tridentate*; etc. A common chelating agent is ethylenediaminetetraacetic acid (EDTA). Nitrilotriacetic acid $N(CH_2COOH)_3$ and ethyleneglycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid $(HOOCCH_2)_2NCH_2CH_2OCH_2CH_2OCH_2CH_2N(CH_2COOH)_2$ are used in analytical chemical titrations and to remove ions from solutions and soils. Metal chelates are found in biological systems, e.g., the iron-binding porphyrin group of hemoglobin and the magnesium-binding chlorophyll of plants. Medicinally, metal chelates are used against Gram-positive bacteria, fungi, viruses, etc.
See ammine; sequestration.

Def. 2

ethylenediaminetetraacetonitrile. (EDTAN). $[-CH_2NCH_2CN_2]_2$.
Properties: White, crystalline solid. Melting range 126–132C, bulk d 48.4 lb/cu ft. Slightly soluble in water; soluble in acetone.
Hazard: Toxic by ingestion and inhalation.
Use: Chelating agent and intermediate.

Def. 3

ethylenediaminetetraacetic acid. (EDTA; ethylenebisiminodiacetic acid; ethylenedinitrilotetraacetic acid).
CAS: 60-00-4.
$(HOOCCH_2)_2NCH_2CH_2N(CH_2COOH)_2$. An organic chelating agent.



Properties: Colorless crystals. Decomposes at 240C. Slightly soluble in water; insoluble in common organic solvents; neutralized by alkali metal hydroxides to form a series of water-soluble salts containing from one to four alkali metal cations.
Derivation: (a) Addition of sodium cyanide and formaldehyde to a basic solution of ethylenediamine (forms the tetrasodium salt); (b) heating tetrahydroxyethylethylenediamine with sodium hydroxide or potassium hydroxide with cadmium oxide catalyst.
Use: Detergents; liquid soaps; shampoos; agricultural chemical sprays; metal cleaning and plating; metal chelating agent; treatment of chlorosis; decontamination of radioactive surfaces; metal deactivator in vegetable oils, oil emulsions, pharmaceutical products, etc.; anticoagulant of blood; eluting agent in ion exchange; to remove insoluble deposits of calcium and magnesium soaps; in textiles to improve dyeing, scouring, and detergent operations; antioxidant; clarification of liquids; analytical chemistry, spectrophotometric titration; aid in reducing blood cholesterol; in medicine to treat lead poisoning and calcinosis; food additive (preservative).
Note: A number of salts of EDTA are available with uses identical or similar to the acid. The USP salts are called edetates (calcium disodium, disodium edetates); others are usually abbreviated to EDTA (tetrasodium, trisodium EDTA). Other salts, known chiefly under trademark names, are the sodium ferric, dihydrogen ferrous and a range of disodium salts with magnesium, divalent cobalt, manganese, copper, zinc, and nickel.

Def. 4

APPENDIX D (cont.)

Stedman's
MEDICAL DICTIONARY

25th Edition
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chelate (kē'lāt). 1. To effect chelation. 2. Pertaining to chelation. 3. A complex formed through chelation.

chelation (kē-lā'shŭn) [G. *chēlē*, claw]. Complex formation involving a metal ion and two or more polar groupings of a single molecule; thus, in heme, the Fe^{2+} ion is chelated by the porphyrin ring. C. can be used to remove an ion from participation in biological reactions, as in the c. of Ca^{2+} of blood by EDTA, which thus acts as an anticoagulant.

Def. 5

ethylenediaminetetraacetic acid (EDTA) (eth'il-ēn-dī'ā-mēn-tet-rā-ă-sē'tik). Edetic acid; edathamil; $(HOOC–CH_2)_2N(CH_2)_2N(CH_2–COOH)_2$; a chelating agent used to remove multivalent cations from solution as chelates, and used in biochemical research to remove Mg^{2+}, Fe^{2+}, etc., from reactions affected by such ions. As the sodium salt, used as a water softener, to stabilize drugs rapidly decomposed in the presence of traces of metal ions, and as an anticoagulant; as the sodium calcium salt, used to remove radium, lead, strontium, plutonium, and cadmium from the skeleton, forming stable un-ionized soluble compounds that are excreted by the kidneys.

Def. 6